bio-protocol Preprint

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Cell culture and adipocyte differentiation

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An abbreviated version of this protocol was published in Science Advances in Dec 2020

Enoxacin induces oxidative metabolism and mitigates obesity by regulating adipose tissue miRNA expression

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Detailed protocol

Current protocol: Cell culture and adipocyte differentiation

Materials and Reagents

1. Murine 3T3-F442A, C3H10T1/2, white subcutaneous (9W), or brown (9B) preadipocytes
2. 20 nM insulin (Sigma-Aldrich, catalog number: I6634)
3. 1 nM triiodothyronine (T3) (Sigma-Aldrich, catalog number: T2877)
4. 0.5 mM 3-Isobutyl-1-methylxanthine (Sigma-Aldrich, catalog number: I5879)
5. 1 µM dexamethasone (Sigma-Aldrich, catalog number: D4902)
6. 0.125 mM indomethacin (Sigma-Aldrich, catalog number: I7378)
7. 2.8 µM rosiglitazone (Sigma-Aldrich, catalog number: R2408)
8. Dulbecco's modified Eagle's medium (high glucose) (Life Technologies, Invitrogen™, catalog number: 11965)
9. Fetal bovine serum (FBS) (Thermo Fisher Scientific, catalog number: 12657-029)
10. Pen/Strep (Thermo Fisher Scientific, catalog number: 15140122)

Equipment

1. 37 °C, 5% CO_2 incubator
2. Water bath

Procedure

Notes:

Pre-warm DMEM media (see Recipes) to 37 °C prior to the experiment

A. Seed the cells

1. Seed 1 x 10^5 preadipocytes in a 12 well plate and cultivate in DMEM media (see Recipes) until 90% confluence
2. Change the media every 2 days

B. Adipocyte differentiation (pro-browning stimulus)

Day 0: 1. Once the cells reach 90% confluence, change the media and cultivate for 2 days
Day 2 2. Replace the media for Differentiation media day 2 (see Recipes) and cultivate for 2 days
Day 4 3. Remove the media and replace with Differentiation media day 4 (see Recipes)
Day 6 4. After 2 days, replace the media with Differentiation media day 6 (see Recipes)
Day 8 5. Cells are fully differentiated and ready to use

Recipes

1. DMEM media (supplemented with 10% FBS and 1% of Pen/Strep)
2. Differentiation media day 2: DMEM media further supplemented with Insulin, Dexamethasone, IBMX, T3, Indomethacin, Rosiglitazone (final concentrations in Material and Reagents)
3. Differentiation media day 4: DMEM media further supplemented with Insulin, T3 and Rosiglitazone (final concentrations in Material and Reagents)

4. Differentiation media day 6: DMEM media further supplemented with Insulin and Rosiglitazone (final concentrations in Material and Reagents)

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Representative images

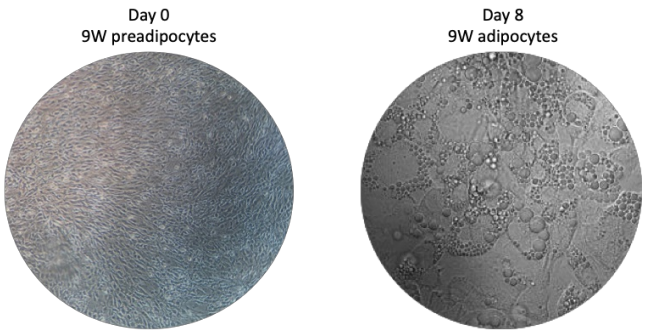

Figure 1. Representative microscopic images of 9W preadipocytes before and after differentiation. Image of 9W preadipocytes at day 0 - 100% confluence and at day 8 - fully differentiated adipocytes.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Mori, M. A. and Rocha, A. L.(2020). Cell culture and adipocyte differentiation. Bio-protocol Preprint. bio-protocol.org/prep712.
2. Rocha, A. L., Lima, T. I. D., Souza, G. P. D., Corrêa, R. O., Ferrucci, D. L., Rodrigues, B., Lopes-Ramos, C., Nilsson, D., Knittel, T. L., Castro, P. R., Fernandes, M. F., Martins, F. D. S., Parmigiani, R. B., Silveira, L. R., Carvalho, H. F., Auwerx, J., Vinolo, M. A. R., Boucher, J. and Mori, M. A.(2020). Enoxacin induces oxidative metabolism and mitigates obesity by regulating adipose tissue miRNA expression. Science Advances 6(49). DOI:

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